Moringa oleifera f-sand Filters for Sustainable Water Purification

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Abstract The purpose of this article is to determine parameters for the design of a *Moringa* seed sand filter for water purification. *Moringa oleifera* seeds containing cationic antimicrobial proteins have been used as natural coagulants for turbidity removal; however, low removal efficiency and high residual organic levels limit their applications. In this work *Moringa* seed extracts were used to reverse the charge of sand (*f*-sand) to +10 mV at a seed dosage of 5.6 g seed/m^2 sand. This *f*-sand filter demonstrated ~4 log removal of 1 μm polystyrene particles and > 8 log removal of *E. coli*. compared to <0.1 log removal for bare sand. Enhanced removal for particles and *E. coli* was dominated by attractive electrostatic interactions. Clean bed filtration modeling predicts a sticking coefficient (α) of 0.8 for *f*-sand compared to 0.01 for bare sand. This α was further validated under a wide range of filtration conditions. Preliminary scale up analyses suggest a point-of-use *f*-sand filter that requires very low amount of seeds annually. The outcome of this work presents the scientific basis for the design of a water purification solution for developing regions, requiring only locally available resources and no use of synthetic chemicals or electricity.

Introduction

The World Health Organization (WHO) has reported that 2.2 million deaths annually were caused by waterborne diseases in 2016[1]. The employment of point-of-use technologies for household level water purification reduces diarrheal disease by 30-40%;[2,6] proposed techniques include boiling, chlorination, biosand filters and ceramic filters.[3-5] Nevetheless their application in developing areas remains limited due to the inaccessibility of available raw materials. Therefore, it is critical to provide water purification technologies that are affordable, effective and derived from locally produced materials to provide safe potable water in the developing world.

Moringa oleifera grows widely in many equatorial regions of the world where public health is threathened by unsafe drinking water.[7-9] The seed contains cationic and antimicrobial proteins (MOCP), which comprise 1.2% of the total protein, and are readily soluble in water[14]. The antimicrobial properties are due to the presence of a helix-loop-helix motif that causes fusion of inner and outer cell membranes[14]. Because of these unique properties, MOCP has been used as coagulants/flocculants for tubidity.[10-19] However, its application as a flocculant is challenged by the fouling of treated water over time, as a result of the residual organic matter released from seeds.[10,20] Our previous work showed that MOCP can be adsorbed onto sand via electrostatic attraction creating functionalized sand (*f*-sand): the antimicrobial and flocculating capability of *f*-sand remains comparable to the original seeds while the residual organic matter is eliminated[21]. The application of *f*-sand to point-of-use water purification is still limited due to the following reasons: 1) limited removal (<1 log removal) of pathogens in dilute concentrations (commonly occuring in surface water) by *f*-sand suspensions due to mass transport limitations, 2) lack of optimization of seed dosage and preparation procedures, and 3) no existing design parameters for scale up.

In this study, we determined critical parameters for the design of this *f*-sand filter (**Fig. 1**). *Moringa* seed extract (simply crushed seed), rather than purified protein, was used while seed dosage and filter preparation time were minimized for ease of field applications. The filter performance was tested using model particle and *E.coli* solutions. We applied classic clean bed filtration (CBF) models to determine critical parameters for scale up design, which were validated under a wide range of filtration conditions. A simple saturation model was used to estimate the filter longetivity. The findings enable the rapid and effective design of point-of-use filters when different local materials are available during field applications at various scales.

Materials and Methods

***f*-sand preparation.** Whole unshelled Nicaraguan *Moringa* seeds were ground by a coffee grinder and mixed with deionized (DI) water for five minutes followed by filtration through a 1.5 μm glass fiber filter (Whatman, UK) and a 0.22 μm cellulose acetate filter (Millipore, MA). The seed extract

was mixed with unwashed glass beads (Sigma Aldrich, MO) for five minutes; the supernatant was discarded and the glass beads were rinsed with DI water three times to remove organic residues. The glass bead slurry (*f*-sand) was then used for packing glass columns. Glass beads with sizes ≤106 μm, 212-300 μm and 425-600 μm were used for model development and validation. The specific preparation procedure and details are presented in **Fig. S1**, **Table S1** and **S2**. To study the surface charge of *f*-sand, 3 μm silica oxide particles (5% w/v, Sigma Aldrich, MO) were used as a substitute. Measurements were conducted in 1 mM NaCl using a Zetasizer Nano instrument (Malvern Instruments, UK).

Model contaminants. Green-yellow fluorescent FluoSpheres® polystyrene particles with a diameter of 1 μm (Life Technologies, CA) were used as model particulate contaminants. Particle concentration was analyzed using a FlowSight® Imaging Flow Cytometer (Millipore, MA). *Escherichia coli* strain TG1 (*E. coli*) containing plasmids that express red fluorescent protein (pCA24N-rfp-lasR) were used as model pathogens at an influent concentration of 10^8 colony-forming-unit (CFU)/ml suspended in a 10 times diluted phosphate-buffered saline (PBS buffer, 0.016 M). Culture media chemicals were removed in the cell suspension by rinsing pellets three times with PBS buffer. Culturing details are described in a previous study[22]. A conventional plate counting method was used to quantify CFU. Confocal laser fluorescence microscopy (CLM) was utilized to visualize the *E. coli* attachment onto the sand surface after filtration. Images (20x objective lens) were taken at a laser excitation of 561 nm and emission of 595±50 nm using Nikon Inverted Eclipse Ti2-E System with Nikon $A1R^+$ confocal detector system.

Column experiments. Particle and *E. coli* filtration tests and breakthrough experiments were performed with glass columns (Bio-rad, CA) of either 10 cm or 5 cm length (L) and either 1.5 cm or 1 cm inner diameter (I.D.). The porosity of the packed sand column was gravimetrically determined to be 0.37. The *f*-sand slurry was quickly poured into the glass column and gently mixed by rotation along the length of the column to remove any trapped bubbles. The columns were then packed with DI water overnight and equilibrated with the background electrolyte for 20 pore volumes before switching to appropriate influent solutions prepared in the same background electrolyte. Sterilized water and PBS buffers (10 times diluted) were used for *E. coli* removal experiments while sterilized vials were used to collect effluent samples. A constant flow rate was achieved using a peristaltic pump (Cole-Parmer, IL) with the feed solution entering from the top of the column.

Experimental log removal (pC^*) for particles was calculated using **Eqn 1**:

$$\text{Log}_{10}\ \text{removal}_{exp} = -\log_{10}\left(\frac{N}{N_0}\right) \quad (1)$$

Where N and N_0 are the effluent and influent particle concentrations. To validate α under various ionic strengths, collector sizes and flow rates, filtration experiments were conducted with 1 μm particles (10^6/ml) as the influent. The default conditions were 1 mM NaCl, a flow rate of 1.6 ml/min and collector

diameter of 106 µm. Breakthrough experiments were conducted with 1 µm particles (10^7/ml) as the influent at a flow rate of 0.7 ml/min with the same collector size.

Model calculations Classic clean bed filtration models[23-29] are widely applied to describe the colloidal/ particle deposition and transport in saturated porous media. The extend of deposition can be estimated from the collector efficiency (η_0), defined as the probability of a particle striking a collector given the column specifics and hydrodynamic conditions. **Eqn 2** demonstrates the correlation between log removal and η_0:

$$\ln\left(\frac{N}{N_0}\right) = -\frac{3}{2}\frac{(1-\varepsilon)L\,\alpha\eta_0}{d_c} \quad (2)$$

where d_c is the collector diameter, ε is the column porosity, L is the column length. The model developed by Tufenkji and Elimelech (TE model) was used to calculate theoretical η_0 due to the superior agreement of the predicted values with experimental data[26]. The sticking coefficient, α, describes the probability of a particle sticking to a collector upon collision and has a theoretical range from 0 to 1. The values of α for *f*-sand and bare sand columns were determined using **Eqn 2** and Log removal_{exp} under various column and flow conditions. Predicted log removal values (Log removal_{pred}) were then calculated using **SI-Eqn 1** with a standard deviation calculated using **SI-Eqn 2**. A saturation equation (**SI-Eqn 5**) was used to calculate the fraction (*f*) of sand area occupied by particles at breakthrough. Details are discussed in the **SI**.

Results and discussion

Minimal seed dosage and time for achieving charge reversal of *f*-sand. Here we optimized seed mass per total surface area of sand (g/m^2) by evaluating zeta potentials of 3µm silica oxide particles in 1 mM NaCl at seed concentrations ranging over four orders of magnitude (0.02 to 230 g seed/m^2, 2×10^{-5} to 0.2 g/ml) As shown in **Fig. 1A**, the zeta potential of sand reverses from -42 mV to +10 mV (1 mM NaCl) at a seed dosage of 1-10 g/m^2.These concentrations are comparable to those previously identified[14].We also show (**Fig. S2A**) that a five-minute mixing time for sand and seed extract is sufficient to enable charge reversal by MOCP (compared to 2-5 hour in previous method[21]). Finally, a simple "stick test" was developed to enable field practitioners to determine if charge reversal of the sand was sucessful. Positively charged *f*-sand stuck on the side of the negatively-charged plastic containers (**Fig. S2B and S2C)** compared to regular sand showing no sticking effect.

***f*-sand filters achieved 3 - 4 $\log_{10}$ removal of 1 µm polystyrene particles and >8 log removal of *E.coli*.** The *f*-sand filter achieved 3-4 log removal of 1 µm particles, compared to 0.060 log removal by the bare sand filter (**Fig. 1C**). Polystyrene particles of 1 µm size were chosen because their size and

charge represent many target microbial contaminants (such as coliform bacteria) and the collector efficiency is typically the lowest at this size as predicted by CBF models.[26] As shown in **Fig. S3**, removal was consistent from 2 to 10 pore volumes (0.7-3.5 bed volumes); values were averaged and presented for each specific seed loading in **Fig. 2D**. Increasing the seed dosage from 1.1 to 5.6 g/m^2 resulted in an increase in log removal from 2.9$\pm$0.2 to 3.9 $\pm$0.4 (compared to 0.060$\pm$0.008 log removal by bare sand filters. Further increases in the seed loading did not increase surface charge or particle removal; thus we chose 5.6 g/m^2 seed loading for all subsequent filtration experiments.

We further determined that the *f*-sand filters removed >8 log red fluorescent *E.coli*, as no bacteria was detected in the effluent (1ml sample volume at pore volumes 4, 6 and 8) when the influent concentration was 10^8 CFU/ml. Bare sand only removed 0.05 log *E.coli* under the same flow conditions (**Fig. 2E**). CLSM images (**Fig. 2G**) of *f*-sand after filtration show bacteria (red dots) attached to the *f*-sand surface while little to no bacterial accumulation was observed on bare sand (**Fig. 2F**). Previous Baclight® stain tests have also demonstrated the loss of viability of the bacteria attached to the *f*-sand surface,[21] likely due to the membrane fusion by the MOCP.[14]

In order to confirm the role of electrostatic attractive forces in the polystyrene particle removal in *f*-sand filters, the classic Derjaguin-Landau-Verwey-Overbeek (DLVO) theory (**SI-Eqns 3 and 4**) was used to calculate an attractive energy between *f*-sand (modeled as a flat plate) and a polystyrene particle vs. a repulsive energy barrier between bare sand and the same particle (**Fig. 1C**). This strongly suggests a favorable deposition condition for negatively charged particles and bacteria on *f*-sand and thus a high value for α.

Sticking coefficient (α) was determined to be 0.8 for the *f*-sand filter compared to 0.01 for bare sand filter. Our experimental data confirmed this trend: we calculated an α of 0.83 $\pm$0.08 for the removal of 1 μm polystyrene particle by *f*-sand compared to an α of 0.011$\pm$0.001 for bare sand. We validated the α value of *f*-sand with a series of filtration experiments at a range of ionic strengths, collector sizes, and flow rates,typical for water treatment processes. As shown in **Fig. 3**, the predicted log removals agree well with experimentally measured log removals under various ionic strengths (1-10 mM) typical in natural waters. The model also agrees with experimental data at different collector sizes (106-520 μm); these collector sizes were selected after considering proper scaling between the filter diameter and the collector size from full scale sand filters based on a previous scale-down analysis of granular activated carbon filters (details in **Table S3**).[30] The experimental log removals at various approach velocities (0.13-0.58 mm/s) were also within the range of predicted values, although the variation in average values is large compared to other conditions. These approach velocities are similar or higher than that of a typical slow sand filter, with head losses between 0.1- 0.6 m (calculation presented in **Table S4**), which is appropriate for gravity driven household applications. This agreement strongly suggests that the sticking coefficient determined is robust and *f*-sand surface sustains its ability

to attract and retain particles under a range of conditions. Natural organic matter (NOM) present in surface waters might interact with protein and sand surfaces; future work will further validate sticking coefficient under various NOM types and concentrations.

Experimentally determined 8 log removal of *E.coli* is higher than the predicted value of 4.7. This predicted value is based on an equivalent diameter of 1.7 μm (for *E. coli* that is 1.2 μm in diameter and 3.7 μm in length) [31] and a Hamaker constant of 6.5×10^{-21}J.The lack of agreement is likely due to the larger interaction area between flexible rod-shape bacteria and that of rigid 1 μm polystyrene spherical particles (area calculation presented in **SI**). The length of the bacteria can also enhance the collector efficiency by improved interception; the predicted log removal of a 3.7 μm sphere is calculated to be 10.49. We propose that the α of *f*-sand determined here be a conservative design parameter with the a safety factor, when considering the design of field filters for bacterial removal.

Filter longevity. To estimate the capacity of the filter, we determined a fraction (*f*) of the sand surface area covered by polystyrene particle contaminants (projected area) at an experimentally determined breakthrough point. The breakthrough point occurred when the N/N_0 increased above 0.1. The two pore volume values right before and after breakthrough were averaged and used to estimate the *f* value (using **SI-Eqn 5**). Six repeated runs (**Fig. S4)** indicate a breakthrough pore volume of 350 ± 116, which corresponds to 400-600 ml, generating an *f* value of $4.1\%\pm2\%$. This value can be used for preliminary scale up design and requires further validation with breakthrough experiments at different column scales.

Environmental implications. The outcome of this study provides the scientific basis for a synthetic chemical-free method employing *Moringa* seed extract to enable sustainable drinking water supply in the developing world. Using the α and *f* experimentally determined here, preliminary scale-up analyses were conducted considering a point-of-use household scale (5 people) filter. We required that the filter provide 10 L/day and >4 log removal of 1 μm particles given a heavily contaminated source water (10^4 /100ml) with an appropriate head loss. The analyses (**Table S5**) indicate that an *f*-sand filter, with dimensions of 5 cm diameter and 1 m length (and 3 cm head loss), would require 0.21 kg seed/ year, (a two-year-old *Moringa* tree produces 480 kg seed/year[9]). Similar analyses were conducted for a community-based scale filter (1000 people) (**SI)**. In addition, our calculation shows that the filter longevity was not limited by the sand surface area capacity.

In order to fully implement the proposed filter in the field, we propose to further validate the values of α, *f* and longevity under more complex solution environments, such as various concentrations and types of natural organic matter, colloids and pathogens. Filter regeneration should also be considered for further work. For treating highly turbid source waters, we propose a hybrid column with a regular sand column on top of an *f*-sand column. Furthermore, testing in the field is required before comprehensive environmental implications can be assessed.

Figure 1. *Moringa* seed extract functionalized sand filter (*f*-sand) for enhanced pathogen and colloidal removal. Cationic and antimicrobial proteins in *Moringa* seeds can be readily dissolved and adsorbed onto a sand surface, reversing the charge of the sand particles. These *f*-sand particles can then be packed into a filter with improved hydrodynamics enabling superior log removal of pathogens. This enhanced sand filter is based on locally available materials and provides an attractive water sanitization option for resource-poor settings.

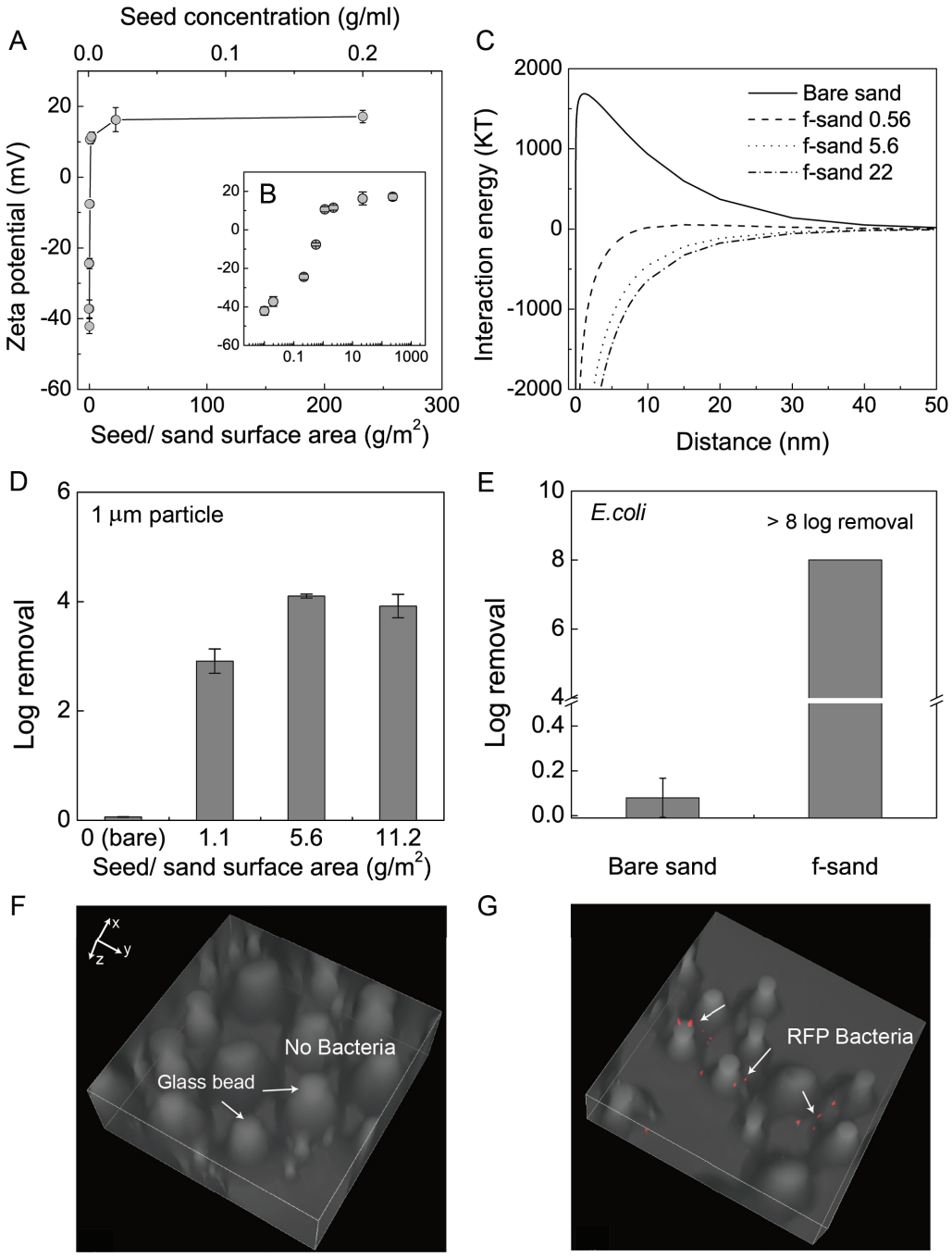

Figure 2. Increasing seed dosage induces charge reversal of *f*-sand while the packed *f*-sand filter achieves ~4 $\log_{10}$ removal of 1 µm polystyrene **particle and >8 $\log_{10}$ removal of *E.coli*. (A)** Zeta potential measurements of *f*-sand indicate charge reversal from -42 mV to +16 mV with increased seed loading. The bottom x-axis represents the seed per sand surface area while the top x-axis shows the corresponding seed concentration. 3 µm silica particles were used as a surrogate for sand and were mixed with moringa serum for 5 min before the measurments (triplicates using 1 mM NaCl as the background electrolyte). **(B) (inset to a)** Identical zeta potential data from A plotted on a logarithm scale. **(C)** DLVO theory based interaction energy calculations suggest strong attractive forces between negatively charged polystyrene particles and the surface of *f*-sand with various seed dosages, in comparison to a large energy barrier seen in bare sand. Values were calculated considering a particle size of 1µm in 1 mM NaCl and a Hamaker constant of 1×10^{-20} J. **(D)** 3-log to 4-log removal of 1 µm particle (10^6 /ml) by a *f*-sand filter with various seed loadings (g seed/m^2 sand surface area) compared to < 0.06 log removal by regular glass beads (bare sand). Filtration experimental conditions include:

106 µm glass beads, 1 mM NaCl, 0.13 mm/s. **(E)** *f*-sand filters removed >8 log *E.coli* compared to 0.05 log removal by bare sand. **(F)** and **(G)** CLSM images of (F) bare sand shows no bacteria attached after filtration for 8 pore volume (2.8 bed volume) of *E.coli* (10^8 CFU/ml), compared to (G) red fluorescent *E.coli* attached to *f*-sand. Light grey contours are glass beads. Bare sand image: x axis: 186.16 µm; y axis: 186.16 µm; z axis: 60.80 µm. *f*-sand image: x axis: 269.84 µm; y axis: 269.84 µm; z axis: 60.80 µm.

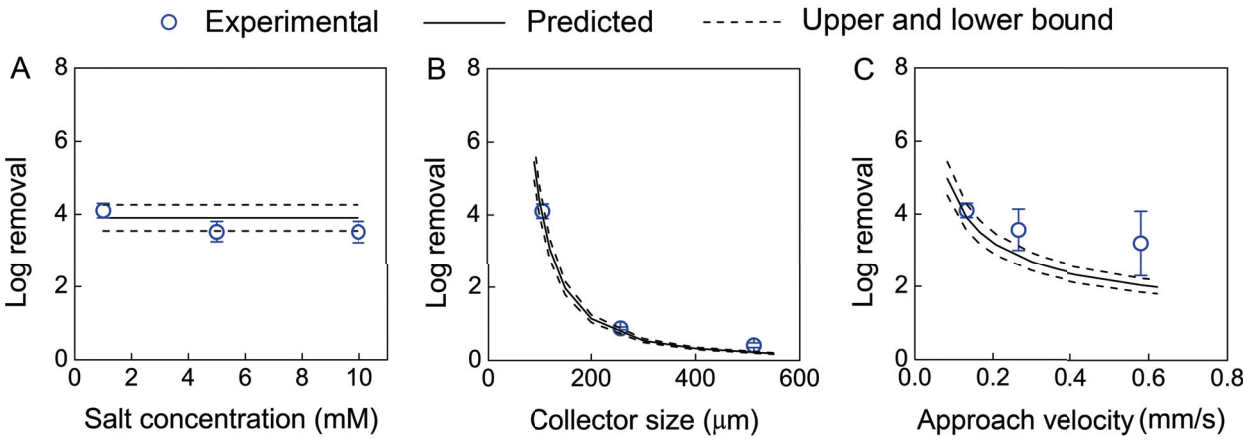

Figure 3. **Experimetal and predicted $\log_{10}$ removal 10^6/ml 1 μm** polystyrene **particles by *f*-sand agrees well** at various **(A)** NaCl concenrtations (1-10mM), **(B)** collector sizes (100-520 μm) and **(C)** approach velocities (0.13-0.58 mm/s, 0.48-2.08 m/h). The predicted removal was calculated using **SI-Eqn 1** and a sticking coefficient of 0.83±0.08 determined from experimental data. The upper and lower bound was generated using the standard deviation calculated using **SI-Eqn 2**. If not specified, the filtration experiments were conducted in 1 mM NaCl with an approach velocity of 0.132 mm/s (1.6 ml/min, 0.48 m/h) with glass beads with collector size of 106 μm at room temperature. All the *f*-sand has a seed loading of 5.6 g/m^2, prepared with 0.005 g/ml seed extract.

ASSOCIATED CONTENT

Supporting Information

The supporting information includes a detailed description of the clean bed filtration model, calculation of interaction energy, head loss and scale up. It also includes additional data for longevity test. The Supporting Information is available free of charge on the ACS Publications website at DOI: xxxx.

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Notes

The authors declare no competing financial interest.

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